Journal of Medical Virology 48:141–146 (1996)

Evaluation of Arthropod-Borne Viruses and Other Infectious Disease Pathogens as the Causes of Febrile Illnesses in the Khartoum Province of Sudan

M.C. McCarthy, R.L. Haberberger, A.W. Salib, B.A. Soliman, A. El-Tigani, I.O. Khalid, and D.M. Watts

U.S. Naval Medical Research Unit No 3, Cairo, Egypt (M.C.M., R.L.H., A.W.S., B.A.S., D.M.W.), Ministry of Health, Khartoum, Sudan (A.E.-T., I.O.K.)

The relative importance of arthropod-borne and other disease pathogens as the cause of an outbreak of febrile illnesses was assessed during August 1988, following severe flooding in Khartoum, Sudan. A total of 200 patients with acute febrile illness and 100 afebrile controls were enrolled in the study during October and November 1988, at the Omdurman Military Hospital, Khartoum, Sudan. Sera were tested for IgM and IgG antibodies to six arthropod-borne viruses by an enzyme-linked immunoabsorbent assay, and for similar antibodies to Lassa fever, Crimean-Congo hemorrhagic fever, and Ebola and Marburg viruses by an indirect fluorescence assay. Thick and thin blood smears were examined microscopically for malaria parasites, and fecal and blood specimens were tested for bacteria by standard culture methods. Among the acute and convalescent sera collected from 67 febrile patients, five cases were caused by sandfly fever Sicilian (SFS), six by sandfly fever Naples (SFN), and 12 by unidentified phleboviruses. Of 233 remaining unpaired, acute-phase sera collected from cases and controls, 49 (21%) had IgM antibodies to SFS or SFN, RVF, West Nile (WN), and Chikungunya (CHIK) viruses. Forty-three (22%) of 192 febrile cases and two of the 100 afebrile controls were positive for *Plasmodium falciparum*, and bacterial enteropathogens were associated with 25 (13%) cases and four controls. These data indicated that phleboviruses and to a lesser extent, WN, *P. falciparum*, and enterobacterial pathogens were causes of acute febrile illnesses following the 1988 flood in Khartoum, Sudan.

© 1996 Wiley-Liss, Inc.*

KEY WORDS: epidemiology, arboviruses, malaria, bacterial diseases, Khartoum, Sudan.

INTRODUCTION

Epidemics and sporadic cases of acute febrile diseases among human populations in Sudan have been associated with infection by several arboviruses including yellow fever (YF), Rift Valley fever (RVF), sandfly fever Sicilian (SFS), sandfly fever Naples (SFN), and dengue (DEN) viruses [Taylor RM et al., 1955; Eisa et al., 1977, 1980; Woodruff et al., 1988; Watts et al., 1994]. However, the relative public health importance of these and other pathogens, such as Ebola, hepatitis, bacterial entero-pathogens, and malaria species, is largely unknown [Bowen ET et al., 1977; Hyams KC et al., 1986; CDCP, 1989].

A major outbreak of acute febrile illness occurred following heavy rains during August 1988, in the Khartoum Province of Sudan. Subsequent flooding of the city of Khartoum and surrounding area by the Blue and White Nile rivers disrupted the shelters and polluted the sources of potable water for approximately one million persons [CDCP, 1989]. During the weeks following the flooding, the Centers for Disease and Prevention (CDC&P) reported a marked increase in cases of acute febrile illness among the displaced persons. Although malaria was associated with a significant number of the cases, the CDC&P also reported that the etiology was unknown for 52–79% of the cases. Therefore, the investigation described in this report was conducted to determine the etiologies and relative importance of arboviral and other infectious diseases as the cause of febrile illnesses in Khartoum, Sudan.

MATERIALS AND METHODS

Study Site and Population

The survey was conducted during October and November 1988, at outpatient medical clinics of the Omdurman Military Hospital in Khartoum, Sudan. These clinics provide tertiary referral services to military per-

Accepted for publication August 15, 1995.

Address reprint requests to Michael C. McCarthy, M.D., who is now at 1755 Park Road, N.W., Washington, D.C. 20010.

© 1996 WILEY-LISS, INC. *This article is a US Government work and, as such, is in the public domain in the United States of America.

sonnel and the general public, including both adult and pediatric populations.

A case of acute fever was defined as any person with a temperature of 100° F or greater upon presentation and a history of fever of no more than 5 days duration. Patients with an obvious focus of infection, such as an abscess, were excluded from the study. Patients who did not have a fever were invited to participate as controls. After providing voluntary informed consent, all patients completed a questionnaire administered by a trained Sudanese physician and underwent a complete physical examination. Cases were requested to return after 2 weeks for follow-up evaluations.

Bacteriology

Clinical specimens for bacteriologic cultures, including stool, urine, and venous blood, were requested from each study subject. Also, a sputum sample was requested from patients with a suspected acute lower respiratory tract infection. Specimens were processed and assayed, and bacterial species were identified according to methods described previously [Hyams et al., 1986].

Parasitology

Two thick and thin blood smears were prepared for each study subject, stained with Giemsa and examined under light microscopy for *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae.*

Virology

Enzyme-linked immunosorbent assays (ELISA) were utilized to test sera for IgM and IgG antibodies to arboviruses, including Chickungunya (CHIK), sandfly fever Sicilian (SFS), sandfly fever Naples (SFN), Rift Valley fever (RVF), West Nile (WN), and Dengue 2 (DEN-2) viruses. A capture assay using goat anti-human IgM antibody bound to 96-well microtiter plates, and antimouse IgG conjugated horseradish peroxidase and a chromogenic substrate (ABTS) were used to test sera for IgM antibody [Summers et al., 1984]. An indirect sandwich assay employing viral infected cell lysates bound to 96-well microtiter plates and a goat anti-human IgG conjugated with horseradish peroxidase and a chromogenic substrate (ABTS) were used to test sera for IgG antibody [Voller et al., 1976]. Each serum sample was diluted in phosphate-buffered saline containing 5% nonfat dry milk and tested initially at 1:100 dilution in duplicate wells. All antibody positive sera were tested at 1:200 through 1:3,200 dilutions to determine titers. Viral specific IgM and IgG antibody positive and negative control sera were used at a 1:100 dilution to verify the validity of all test results. The optical density (OD) of the content of the microtiter plate wells were read at 414 nm wavelength using a spectrophometer. Antigen-free wells were included to obtain baseline OD values for comparison with the values for wells that contained the viral antigens included for the actual testing of sera for antibodies. The OD values for the well without antigen were subtracted from those of the wells with antigen to yield corrected absorbance values. Sera dilutions with corrected absorbance values greater than the reference cut-off value, estimated as the mean absorbance of 10 antibody negative sera, plus three standard deviations were considered antibody positive.

The criteria for a specific viral etiology of an acute case of febrile illness required a fourfold or greater increase in IgM antibody titer between the acute and convalescence phase of illness serum samples. A presumptive diagnosis of a case was the detection of IgM antibody at a 1:200 or greater titer in sera obtained from cases only during the acute phase of illness. Also, the demonstration of a fourfold or greater increase in only IgG antibody titer to antigenically related SFS, SFN, and RVF viruses was considered as evidence of either infection by one or more of these viruses, or possibly by unidentified antigenically related phlebovirus(es).

All sera were tested at a 1:10 dilution for IgM and IgG antibodies to Crimean-Congo hemorrhagic fever (CCHF), Marburg, Lassa, and Ebola viruses by indirect fluorescence as described previously [Wulff and Lang, 1975]. Composite slides, including irradiated antigens for each of the four viruses, and antibody negative and positive control sera were provided by the United States Army Medical Research Institute of Infectious Diseases, (Ft. Detrick, Frederick, MD).

Other

A complete blood cell count (CBC) employing a Coulter Counter with microscopic differential was carried out on all blood specimens. Analysis of urine specimens was carried out by standard procedures.

RESULTS

A total of 200 cases of acute febrile illnesses and 100 afebrile controls were enrolled into the study; 74% (n = 147) were males. The mean age of cases was 20 years (range 1–89 years). All subjects had resided in Khartoum and none had travelled outside the Khartoum province during the month prior to enrollment in the study. At the time of presentation, 23% of cases reported that they were taking antimalarial drugs and 60% reported taking antibiotics.

The mean number of days of fever reported by cases was 3 ± 1 days. The symptoms described most frequently were headache (83%), myalgia or arthralgia (81%), backache (63%), chills (53%), and abdominal discomfort (48%). A history of diarrhea was related by only 9% of cases, and 21% reported that another family member was ill with fever at the time of enrollment, as compared to only 4% of the controls $P < 0.001$. At the time of presentation, 67% of the patients were assessed as being mildly ill and 33% ranged from moderately to severely ill.

Among 192 of the 200 cases and all 100 controls, 43 (22%) of the cases and 2 of the controls were positive for *P. falciparum,* ($P < 0.001$). All patients were negative for *P. vivax, P. ovale,* and *P. malariae.*

TABLE I. Serological Diagnosis of Sandfly Fever Sicilian and Naples Viral Infections Among Febrile Cases of Illness During 1988 in Khartoum, Sudan

	Enzyme-linked immunoabsorbent antibodies			
	Sandfly fever Sicilian		Sandfly fever Naples	
Patient number	IgM Acute/conval[a]	IgG Acute/conval	IgM Acute/conval	IgG Acute/conval
006[b]	<100/1600	100/1600	Neg	200/400
043[b]	<100/800	<100/1600	Neg	Neg
073[b]	<100/800	200/≥3200	Neg	Neg
126[b]	<100/1600	800/≥3200	Neg	1600/800
136[b]	<100/800	800/≥3200	NT[c]	NT
009[d]	Neg	≥3200/≥3200	>100/≥3200	800/≥3200
013[d]	Neg	100/200	100/≥3200	1600/≥3200
080[d]	Neg	200/1600	<100/800	≥3200/≥3200
097[d]	Neg	800/400	<100/≥3200	<100/≥3200
153[d]	Neg	200/400	<100/≥3200	<100/≥3200
164[d]	Neg	≥3200/≥3200	<100/≥3200	<100/≥3200

[a]Acute/convalescent phase of illness sera.
[b]Sandfly Sicilian fever cases.
[c]Sera not tested.
[d]Sandfly Naples cases.

Stool and/or blood cultures from 13 (7%) of 193 cases and from one (1%) of 100 controls ($P = 0.06$) were positive for *S. typhi* or *S. paratyphi*. Stool samples from nine cases and from one control were positive for *Shigella* species, and *Campylobacter* species were isolated from three cases and two controls.

Among 67 paired acute and convalescent serum samples obtained from cases, a fourfold increase in antibody titer indicated that five cases were caused by SFS and six by SFN virus (Table I). An additional 12 (18%) patients had a fourfold rise in only the IgG antibody titer to one or more of the three antigenically related phleboviruses (SFS, SFN, or RVF). These results were likely to reflect infection of individuals with preexisting heterologous antibody by one or more of the latter viruses, or perhaps one or more unidentified phlebovirus. Among the 11 cases of SFS and SFN, sera from two were reactive at dilutions ranging from 1:200 to 1:400 for RVF IgM antibody (Table I). However, a fourfold increase in RVF IgM antibody was not demonstrated, and none of the patients were positive for IgM antibodies to WN, DEN-2, or CHIK viruses.

Of the remaining 133 cases from whom only an acute phase of illness serum was obtained, 16 (12%) had presumptive evidence of a phleboviral infection as indicated by IgM antibodies to SFS (5%) and SFN (8%) viruses. Among the other viruses considered, reactivity was demonstrated for IgM antibodies to CHIK (0.8%), RVF (5%), WN (4%) viruses, and all were negative for DEN viral IgM antibody.

Among the 11 confirmed SFS and SFN cases and the 12 patients infected with unknown phleboviruses, two were also positive for *P. falciparum*, and four for *S. typhi* or *S. paratyphi* infection. Epidemologic, historical, clinical, or laboratory information did not reveal any parameters that differentiated acute phleboviral infection from either malaria or enteric fever in this study population.

The overall frequency of different arboviral antibodies among 196 cases and 100 control study subjects is presented in Table II. The rates for IgM antibodies ranged from 0.0% for DEN to a maximum of 8.0% for SFN and RVF viruses, and the rates for IgG ranged from 10% for CHIK to 60% for WN virus. The frequency of IgM and IgG antibodies, whether detected alone or in combination from a study subject, were comparable among the cases and controls. A low prevalence of IgG antibody was demonstrated for Lassa (n = 6), CCHF (n = 5), and Marburg (n = 1) viruses, and all study subjects were negative for Ebola viral antibody.

Age stratification of the prevalence data revealed that infection by WN, DEN-2, SFS, SFN, and RVF increased significantly with age in this study population (Fig. 1). However, infection appeared to be highest during childhood and adolescence. Sex stratification of the prevalence data suggested that flaviviral infections was significantly higher ($P < 0.001$) among men as compared to women 70% (151/214) vs. 49% (42/86), respectively.

DISCUSSION

Epidemics of acute febrile illness have been reported to occur following heavy rainfalls in areas where arboviral pathogens are endemic [Bissel, 1983]. The clinician's ability to distinguish between bacterial, parasitic, and viral infections during such outbreaks is often difficult [Hyams et al., 1986]. Diagnostic dilemmas are further complicated by a lack of medical technology in developing countries, such as Sudan. Consequently, the relative clinical importance of different infections during epidemic conditions is frequently unknown.

Observations made during this study indicated that acute malaria continued to be a cause of febrile illness several weeks following the initial flooding. The significantly greater proportion of cases infected with *P. falciparum* as compared to the controls suggested that

TABLE II. Summary of Arboviral and Other Viral Antibodies Demonstrated Among Sudanese During 1988 in Khartoum, Sudan

	Cases (n = 196)		Controls (n = 100)	
Viruses[a]	IgM	IgG	IgM	IgG
Chikungunya	0.5[b]	10	1.0	11
Sandfly Sicilian	6.0	54	7.0	53
Sandfly Naples	8.0	34	5.0	35
Rift Valley	8.0	20	4.0	22
West Nile	3.0	60	7.0	57
Dengue	0.0	48	0.0	49

[a]Reactivity was demonstrated for IgG antibodies to Lassa (2.0%), Crimean-Congo hemorrhagic fever (2.0%), and Marburg (0.3%) viruses. Not included are data for 12 study subjects infected by an undetermined phlebovirus (es).
[b]Percent.

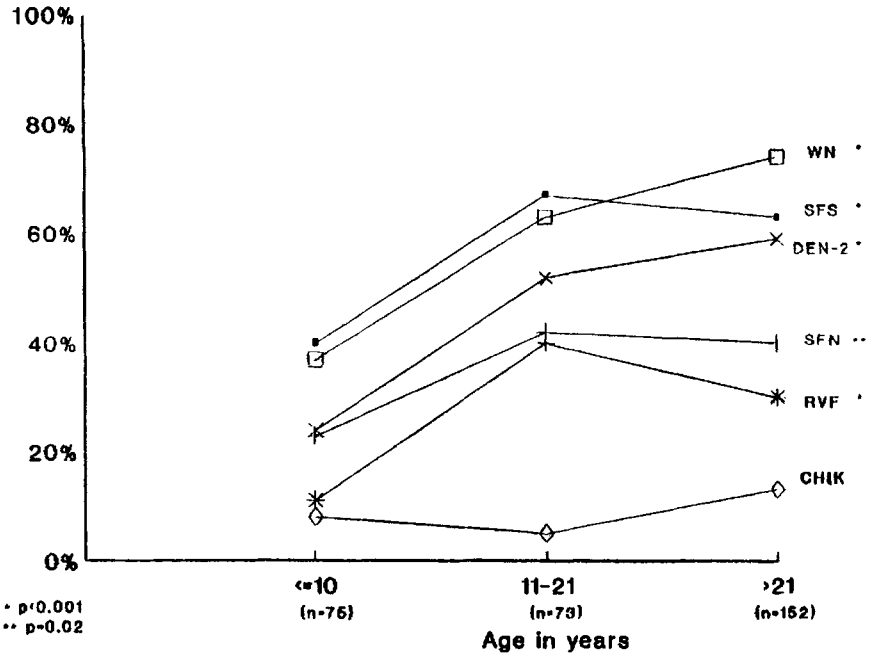

Fig. 1. Age-specific prevalence of total (IgG/IgM) antibody to selected arboviral pathogens.

malaria transmission in this region was epidemic rather than endemic. The CDC&P reported similar results from Khartoum during the weeks immediately following the floods [CDCP, 1989]. That *P. falciparum* was the predominant cause of acute malaria during this study and that other *Plasmodia* species were not associated with cases were consistent with data reported from Port Sudan, which showed that *P. falciparum* was also the predominant cause of acute malaria. However, cases were also caused by *P. vivax* and *P. malariae* in Port Sudan [Hyams et al., 1986]. These observations emphasize the relative clinical importance of *P. falciparum* as compared to other *Plasmodia* species, as well as highlights the geographic differences in the epidemiology of malaria in Sudan.

The proportion of cases infected with *Salmonella* and *Shigella* species were markedly higher than for the controls, thus suggesting that asymptomatic infection with enteric pathogens was uncommon during this study. The results also indicated that infection with enteric pathogens was uncommon in this study population. In contrast, the CDC&P reported that diarrheal disease accounted for 38% of total morbidity among children during the weeks immediately following the floods [CDCP, 1989]. Also, results reported during an outbreak in Port Sudan indicated that nearly 20% of the cases of acute febrile illness were diagnosed as having enteric fever caused by *Salmonella* species [Hyams et al., 1986]. One possible explanation for the lower rate of enteric diseases during this study was the establishment of sources of potable water prior and during the time that this investigation was conducted in Khartoum.

Our data revealed that dual infection by *P. falciparum* or *Salmonella* species was found among six of 23 subjects with acute phleboviral infection. Similar find-

ings were reported among patients with acute dengue fever in Port Sudan [Hyams et al., 1986]. In both studies, arboviral infections were clinically indistinguishable from malaria or enteric fever. These results emphasize the clinical challenge in establishing diagnoses for infectious diseases in developing countries, such as Sudan.

The results of this study supported previous observations that associated several arboviruses with outbreak of disease in Sudan [Eisa et al., 1977, 1980; Hyams et al., 1986; Woodruff et al., 1988; Watts et al., 1994]. Among the viruses considered during the outbreak of febrile illness in Khartoum, phleboviruses were the most frequent cause of morbidity. These viruses were also associated with an outbreak of human disease along the Nile River in Northern Sudan [Watts et al., 1994]. However, previous data reported for Port Sudan indicated that dengue was the most common cause of febrile illnesses [Hyams et al., 1986]. The results of these studies underscore the importance of acute arboviral infections as well as highlights the wide geographical distribution and variation of arboviral pathogens in Sudan.

The proportion of cases infected with phleboviruses, WN, and CHIK were comparable to that of the controls, thus suggesting that asymptomatic infections may have been caused by these viruses. An alternative possibility was that either a symptomatic or asymptomatic infection occurred prior to this study and therefore induced the IgM antibody that persisted at detectable levels for several months. However, since a follow-up serum sample was not available, a possible increase in antibody titer could not be determined as an indication of a diagnosis.

Age-stratified prevalence data indicated that arboviral infections among residents of Khartoum appeared to be highest during the first 20 years of life and that infection continues to increase with age. Whereas phleboviral infections were more common during this outbreak, the demonstration of IgG antibody for DEN and/or WN in 49% or more of the study subjects indicated that previous human infections by one or both of these viruses were common in Khartoum. Although DEN virus was not associated with acute cases during this study, IgM antibody as evidence of WN Nile infection was demonstrated in both the cases and controls. These results implied that DEN viruses were not transmitted during the present investigations, even though the data suggested that this virus was endemic in Khartoum. The demonstration of antibody to RVF virus was an important finding because outbreaks of this disease in Egypt have been linked to livestock imported from the Sudan [Gad et al., 1986]. However, the identification of viral antibodies detected by the ELISA must be interpreted with caution because of possibility of cross-reactivity among antigenically related arboviruses [Calisher et al., 1988]. A very low prevalence of antibodies to other hemorrhagic fever viruses (CCHF, Marburg, and Lassa) was found in this study. These results were consistent with those reported from Port Sudan [Hyams et al., 1986]. The low prevalence of antibodies to these viruses, however, may reflect the high case fatality rates associated with infection rather than the actual prevalence in this region.

In conclusion, the results of this study indicated that acute febrile illnesses were caused primarily by falciparum malaria, bacterial enteropathogens, and phleboviruses following the flood during 1988 in Khartoum, Sudan. Arboviral infections were clinically indistinguishable from acute falciparum malaria and/or enteric fever. Also, the results of this and other studies indicated that several arboviruses were endemic and that there was significant geographic variation in the epidemiology of both arboviruses and malaria species in the Sudan.

ACKNOWLEDGMENTS

This work was supported by the Naval Medical Research and Development Command, NMC, NCR (Bethesda, MD), Work Unit No. 3M162770.A870.AR.322. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department, Department of Defense, or the U.S. Government.

REFERENCES

Bissel RA (1983): Delayed-impact of infectious disease after a natural disaster. Journal of Emergency Medicine 1:59–66.

Bowen ET, Platt GS, Lloyd G, Baskerville A, Harris WJ and Vella EE (1977): Viral hemorrhagic fever in Southern Sudan and Northern Zaire: Preliminary studies on the aetiological agent. Lancet 1:571–573.

Calisher CH, Karabatsos N (1988): Arbovirus serogroups: Definition and geographic distribution. In Monath TP (ed): "The Arboviruses-Epidemiology and Ecology, Vol. 1." Boca Raton: CRC Press, pp 19–57.

Centers for Disease Control and Prevention (CDCP) (1989): Health Assessment for the population affected by flood conditions—Khartoum, Sudan. Morbidity and Mortality Weekly Report 37:785–788.

Eisa M, Obeid HMA, El Sawi ASA (1977): Rift Valley Fever in the Sudan: I—Results on field investigations of the first epizootic in Kosti District, 1973. Bulletin of Animal Health and Production in Africa 24:343–347.

Eisa M, Kheir El Sid ED, Shomein AM, Meegan JM (1980). An outbreak of Rift Valley fever in the Sudan, 1976. Transactions of the Royal Society of Tropical Medicine and Hygiene 74:417–419.

Gad AM, Feinsod FM, Allam IH, Eisa M, Hassan AN, Soliman BA, El Said S, Saah AJ (1986). A possible route for the introduction of Rift Valley fever virus into Egypt during 1977. Journal of Tropical Medicine & Hygiene 89:233–236.

Hyams KC, Oldfield EC, Scott RM, Bourgeois AL, Gardiner H, Pazzaglia G, Moussa M, Saleh AS, Dawi OE, Daniell FD (1986): Evaluation of febrile patients in Port Sudan, Sudan: Isolation of dengue virus. American Journal of Tropical Medicine and Hygiene 35: 860–865.

Satti MH (1966): An outbreak of yellow fever in the Southern Fung and Upper Nile province, Republic of the Sudan. Journal of Tropical Medicine & Hygiene 69:36–44.

Summers PL, Eckles KH, Dalrymple JM, Boyd VA (1984): Antibody response to dengue 2 vaccine measured by 2 different radioimmunoassay methods. Journal of Clinical Microbiology 19:651–653.

Taylor RM, Haseeb MA, Work TH (1955). A regional reconnaissance on yellow fever in the Sudan with special reference to primate hosts. Bulletin of the World Health Organization 12:711–725.

Voller A, Bidwell D, Bartlett A (1976). Microplate Enzyme immunoassays for the immunodiagnosis of virus infections. In: Rose NR and Friedman H (eds): "Manual of Clinical Immunology." Washington, DC: American Society for Microbiology, pp 506–512.

Watts DM, El-Tigani A, Botros BAM, Salib AW, Olson JG, McCarthy M, Ksiazek TG (1993). Arthropod-borne viral infections associated

with a fever outbreak in the Northern Province of Sudan. Journal of Tropical Medicine & Hygiene 97:228–230.

Woodruff PWR, Morrill JC, Burans JC, Hyams KC, Woody JN (1988): A study of viral and rickettsial exposure and cases of fever in Juba, Southern Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene 82:761–766.

Wulff H, Lang JV (1975): Indirect immunofluorscence for the diagnosis of Lassa fever infection. Bulletin of the World Health Organization 52:429–436.

Wulff H, Johnson KM (1979). Immunoglobulin M and G responses measured by immunofluorscence in patients with Lassa or Marburg virus infections. Bulletin of the World Health Organization 57:631–635.